Invasive Pulmonary Aspergillosis Successfully Treated with Granulocyte Transfusions Followed by Hematopoietic Stem Cell Transplantation in a Patient with Severe Childhood Aplastic Anemia

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Granulocyte transfusions (GTX) have been used in patients with neutropenia or neutropenia associated with invasive fungal infection. An 11-year-old girl with severe aplastic anemia (SAA) received immunosuppressive therapy (IST) with rabbit antithymocyte globulin, cyclosporine, and granulocyte colony-stimulating factor. However, IST was not effective and her condition became complicated with life-threatening invasive pulmonary aspergillosis. Owing to the necessity for early neutrophil recovery to resolve the infection, GTX were performed, followed by bone marrow transplantation (BMT) from her mother with human leukocyte antigen-B locus mismatch. Her dyspnea improved and she eventually became afebrile after the initiation of GTX.Despite engraftment failure following BMT, successful engraftment was achieved by salvage therapy with peripheral blood stem cell transplantation. Chest computed tomography scan obtained 4 months after BMT revealed marked improvement in pneumonia. The current case illustrates that GTX may be useful in controlling invasive fungal infections before hematopoietic stem cell transplantation in patients with SAA.

Key words: invasive pulmonary aspergillosis, granulocyte transfusion, hematopoietic stem cell transplantation, childhood severe aplastic anemia

INTRODUCTION

In patients with severe aplastic anemia (SAA), controlling the invasive fungal infection (IFI) could be difficult, particularly during the neutropenic period. Granulocyte transfusions (GTX) can serve as a therapeutic alternative in patients with SAA having life-threatening IFI [1, 2]. Several clinical studies have also reported the clinical benefit of GTX in hematopoietic stem cell transplantation (HSCT) recipients with severe bacterial or fungal infections [3, 4]. Herein, we report the case of a pediatric patient with SAA complicated by life-threatening invasive pulmonary aspergillosis (IPA) that was successfully treated with GTX, followed by HSCT.

CASE

An 11-year-old girl was admitted to our hospital for pancytopenia. Physical examination revealed facial pallor and purpura. Hepatosplenomegaly was absent. Laboratory findings were as follows: leukocytes, 600/μL; neutrophils, 2%; hemoglobin, 5.4g/dL; and platelets, $0.6 \times 10^4/\mu$L. Bone marrow examination showed hypoplasia (96% lymphocytes) and fatty marrow. Cytomegalovirus (CMV) antibody was negative.

On the basis of these findings, the patient was diagnosed with SAA.

Immunosuppressive therapy with rabbit antithymocyte globulin, cyclosporine, and granulocyte colony-stimulating factor (G-CSF) was initiated owing to the absence of human leukocyte antigen (HLA)-identical relative donor. However, no signs of hematologic recovery were noted and severe leukocytopenia persisted. Although prophylactic micafungin (1mg/kg) was initiated, the level of serum Aspergillus antigen was elevated, resulting in the diagnosis of probable Aspergillus infection. She also developed IPA (Figure A). Despite treatment with liposomal amphotericin B (L-AMPL) instead of voriconazole owing to the elevated transaminase levels, pneumonia progressed, which was concomitant with high fever and dyspnea. To control IFI, we decided to treat her with GTX, followed by bone marrow transplantation (BMT) from her mother with HLA-B locus mismatch. Her ABO-matched parents, both of whom were positive for CMV antibodies, were the donors for GTX. The institutional review board of Showa University Fujigaoka Hospital approved the correction of granulocytes using G-CSF for healthy donors. Informed consent for G-CSF mobilization was also obtained from her parents. Each donor was administered G-CSF at 5 μg/kg s.c. the night before granulocyte collection. Granulocytapheresis was performed on the COBE Spectra blood cell separator using 7 L of blood with continuous flow centrifugation. The granulocytes were

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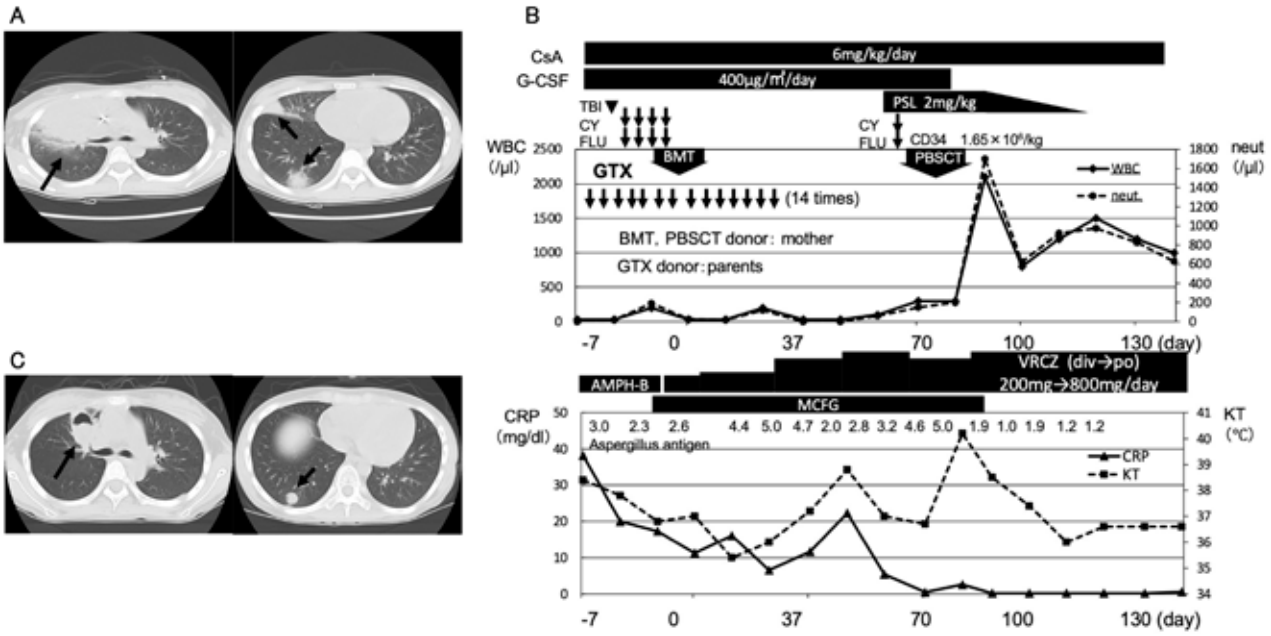

Figure

A. Chest computed tomography: Before the granulocyte transfusions (GTX), pulmonary infiltrates and pleural masses were observed in her right lung (arrows).
B. Clinical course associated with hematopoietic stem cell transplantation (HSCT) and GTX
C. Chest computed tomography: 4 months after bone marrow transplantation, pulmonary infiltrates and pleural masses showed dramatic improvement (arrows)

collected three and four times from her father and mother, respectively. The total number of collected cells was 7 (5–12) × 10^{10}, which was divided into two parts with irradiation at 25 Gy. An average dose of 0.8 × 10^9 granulocytes/kg was transfused. The donor showed tolerance to the procedure without any serious side effects due to prophylactic prednisolone (1mg/kg) and acetaminophen (10mg/kg) administered before GTX. GTX were initiated 7 days before BMT in addition to the antifungal therapy. The conditioning therapy included fludarabine (25mg/m^2/day for 4days), cyclophosphamide (CY) (750mg/m^2/day for 4days), and total body irradiation (2 Gy/day for 1 day) (Figure B). In 1 h, 3.5 (2.5–6) × 10^{10} granulocytes were transfused to this patient. Corticosteroids and acetaminophen were simultaneously administered, and she showed adequate tolerance to the procedure. Her dyspnea showed improvement and she eventually became afebrile after the initiation of GTX. C-reactive protein level also showed a dramatic decline (from 40mg/dl to 10mg/dl). Following this, bone marrow cells (2.27 × 10^8/kg nucleated cells) were transplanted into the patient. The patient developed an anaphylactic reaction to L-AMPL prior to BMT. Therefore, antifungal therapy was switched to voriconazole and micafungin. On day 21 after BMT, microsatellite chimerism analysis revealed a graft rejection without anti-HLA antibody.

Therefore, peripheral blood stem cell transplantation (PBSCT; 1.65 × 10^6/kg CD34 + cells) from the same donor was performed 37 days after the first BMT concomitant with 1-day conditioning therapy comprising fludarabine (25mg/m^2/day) and CY (750mg/m^2/day). Engraftment was achieved and confirmed by complete donor chimerism 18 days after PBSCT. Her dyspnea resolved, and a chest computed tomography scan obtained 4 months after BMT demonstrated a marked improvement in her pneumonia (Figure C). The patient was positive for CMV antibodies after the HSCT, but CMV antigenemia and infection were not observed. Mild infiltration persisted; however, she was alive for 2 years after HSCT.

DISCUSSION

Life-threatening IFI can sometimes occur in SAA as a result of the extensive neutropenic period of immunosuppressive therapy. HSCT, which is required for patients with SAA in whom immunosuppressive therapy fails to restore bone marrow function, can result in morbidity, particularly in patients with SAA and IFI. West *et al.* reviewed 32 of 46 patients (70%) with proven Aspergillus infection who showed an overall response with GTX in G-CSF era (1993–2005) [1]. In a randomized controlled trial of therapeutic GTX, subjects who received an average dose per transfusion of > 0.6 × 10^9 granulocytes/kg showed better outcomes than those who received a lower dose [2]. An average dose of 0.8 × 10^9 granulocytes/kg was transfused to our patient, which is consistent with the findings of the previous study. Price *et al.* concluded that GTX was favorable for the survival of 19 patients with fungal or bacterial infections who underwent GTX before and after HSCT [3]. Our patient also received GTX before the conditioning therapy. IFI can be controlled with GTX, and BMT can also be safely performed. The reported adverse effects of GTX include allergic reactions, HLA alloimmunization causing engraftment rejection, chronic graft-versus-host disease, and CMV infection acquired from the donor [3, 5, 6]. Although HLA antibodies were absent in the present case, engraftment failure occurred following BMT. However,

successful engraftment was achieved by salvage therapy with subsequent PBSCT. Shigemura *et al*. also reported that a patient with chronic granulomatous disease and IFI who received GTX exhibited engraftment failure after cord blood transplantation despite the absence of anti-HLA antibodies [7]. Takahata *et al*. reported a case of successful engraftment with PBSCT after GTX from the same donor [8]. Although an HSCT donor can be chosen as a GTX donor when the donor pool is limited for GTX, it might be better chosen different GTX donor from HSCT donor. Although the patient in our study did not develop CMV infection, van de Wetering *et al*. reported that CMV infection derived from donors might be a fatal complication [6]. A recent Cochrane review on therapeutic GTX concluded that there is insufficient evidence to determine whether GTX affects mortality in patients who are neutropenic caused by myelosuppressive chemotherapy or HSCT [9], and GTX should be limited to individual uncontrolled cases. In the current patient, who had an uncontrolled condition, GTX with antifungal therapies could have been useful.

Moreover, GTX was useful in our index patient with IFI that could not be controlled with antifungal therapy. Dedieu *et al*. also reported that GTX should be considered before HSCT in patients with uncontrolled IFI for more than 3 months [10]. Although GTX led to the complete cure of IPA, GTX can also be utilized to bridge the gap between engraftment and HSCT. West *et al*. recommended the use of GTX in uncontrolled IFI if it is immediately available in sufficient cell doses (at least 1.0×10^{10} or $> 0.6 \times 10^{9}$ granulocytes/kg) [1].

CONCLUSION

In conclusion, we report the case of uncontrolled IFI in a severely neutropenic pediatric patient with SAA who was successfully treated with GTX, thereby enabling the patient to undergo HSCT. The current case illustrates the utility of this approach in similar scenarios, which requires further investigation.

DECLARATION OF CONFLICTING INTERESTS

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

Our institution does not require ethical approval for reporting individual cases or case series.

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INFORMED CONSENT

Written informed consent was obtained from the patient for their anonymized information to be published in this article.

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